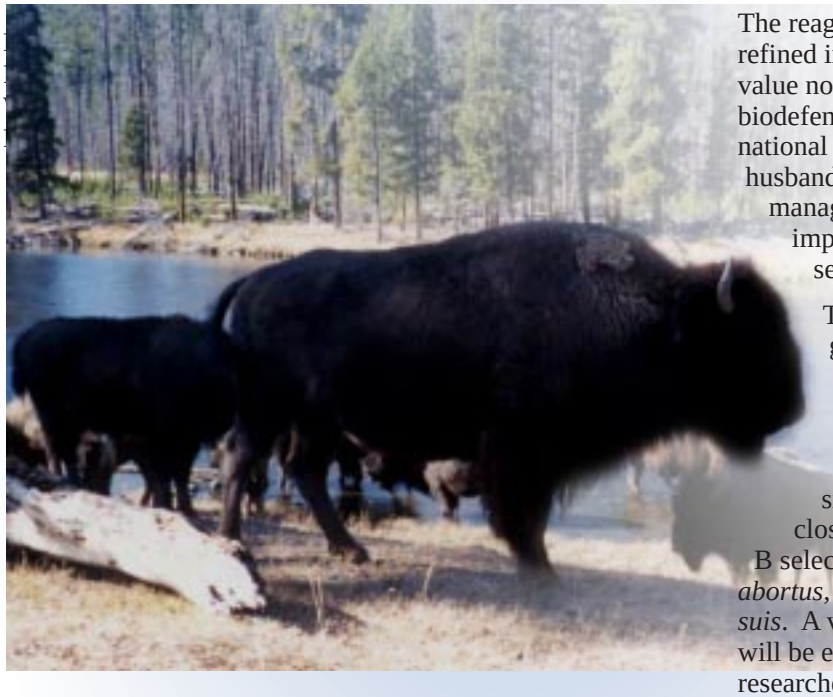
Detection and Forensic Analysis of Wildlife and Zoonotic Disease

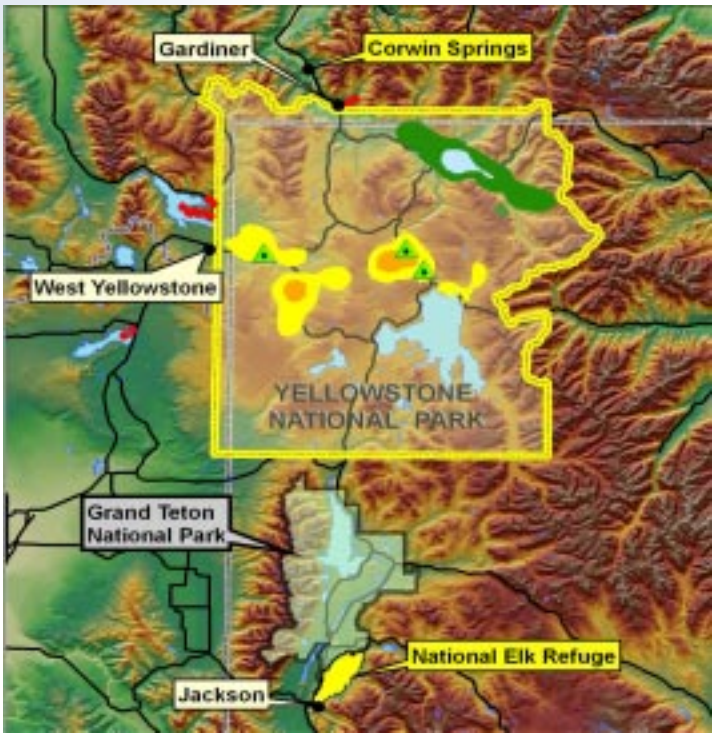


Brucellosis is endemic to the bison and elk populations within the Greater Yellowstone Area.

The INL is developing a variety of assays and techniques that will facilitate detection and molecular fingerprinting of high consequence pathogens. Current work is focused on the detection and forensic analysis of *Brucella* spp., which are animal and human pathogens responsible for disease in a broad spectrum of hosts. Concern exists over the possible use of *Brucella* spp. as agents of biological warfare directed towards humans and/or domestic animals, specifically cattle. In addition, this research will contribute to understanding the potential for natural transmission of brucellosis from bison and elk populations – in which the disease is endemic – domesticated animals (cattle) in the Greater Yellowstone Area.

The reagents developed and refined in our work will have value not only to national biodefense, but also to national and regional animal husbandry, and wildlife management issues that impact U.S. agricultural security.

The project will generate a unique set of validated (against real world diagnostic and environmental samples) DNA signatures for the closely related Category B select agents, *Brucella abortus*, *B. melitensis*, and *B. suis*. A variety of techniques will be employed. INL researchers have developed a real-time (fluorescence-based) Polymerase Chain Reaction (PCR) test that allows detection of active *Brucella abortus* infection in bison, other wildlife, and cattle in approximately 30 minutes. This is an improvement over conventional (gel-based) PCR which typically requires about 3 hours for assay results. INL has a field-portable real-time PCR instrument allowing the assay to be run in the field at trap sites. Additional real-time PCR assays are being developed and validated to target other species, incorporate internal control, and allow multiplexing (detection of more than one target in a single reaction). While real-



Distribution of the northern (green) and central (yellow) bison herds within Yellowstone National Park. Red indicates seasonal migration outside of the park boundaries. Green triangles indicate sites where samples have been taken for real-time PCR and cultivation analyses.

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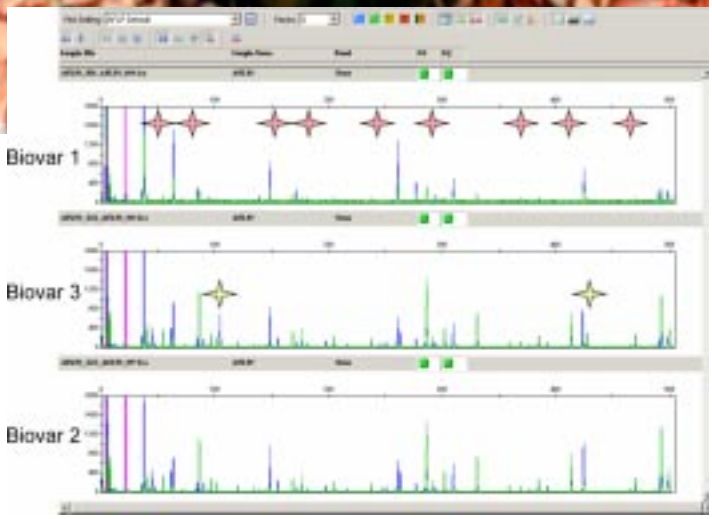
INL is a U.S. Department of Energy national laboratory operated by Battelle Energy Alliance

Field-portable real-time PCR instrument called the Ruggedized Advanced Pathogen Identification Device (RAPID).



B. melitensis *AFLP Analysis. Stars indicate positions of unique fragments with potential for use in high-resolution typing.*

time PCR is rapid and sensitive, it may not afford a suitable platform to perform strain typing, particularly within the *Brucella*, which are genetically very homogeneous across species and strains. Accordingly, scientists are developing appropriate methods and instrumentation to perform rapid, high-throughput microbial forensic analysis of samples for identification at the strain or isolate level. By combining sets of highly-discriminatory primers to amplify and label repetitive or unique sequences from the target organism's DNA, with high-throughput, high resolution capillary electrophoresis, a means of handling large numbers of samples will be established. Strain typing of pathogenic strains by molecular methods is important to epidemiological and forensic studies. Methods include pulsed field gel electrophoresis of large chromosomal restriction fragments, insertion element number and restriction fragment length polymorphisms (RFLP), rRNA RFLP patterns (ribotyping), arbitrary fragment length polymorphisms (AFLP), and analysis of variable-number tandem repeats (VNTR).

Newby, D.T., T.L. Hadfield, and F.F. Roberto. 2003 Real-time PCR detection of *Brucella abortus*: a comparative study of SYBR Green I, 5'-exonuclease, and hybridization probe assays. Appl. Environ. Microbiol. 69:4753-4759.